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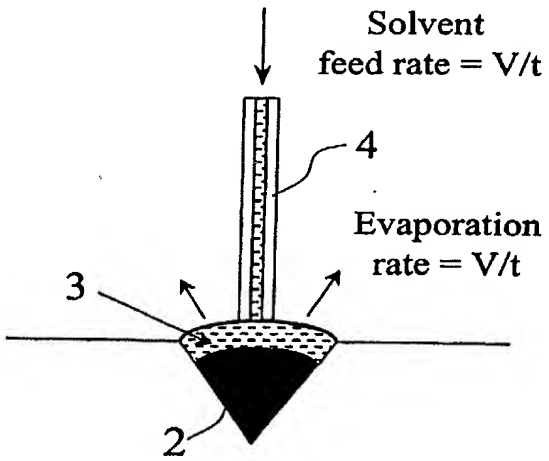

(57) Abstract

The invention relates to a method of preventing evaporation from a small volume liquid sample, especially an ultra-small volume liquid sample, during any handling or chemical operation thereof, which comprises performing said operation with the sample covered by a layer of a liquid which is substantially immiscible with said liquid sample and which is evaporable at such temperature and time conditions at which decomposition of the components of the liquid sampled is avoided. By proper choice of cover liquid it will be evaporated during the operation, additional cover liquid being added to compensate for said evaporation, and easily and rapidly removed when the operation has been terminated.

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A METHOD OF PREVENTING EVAPORATION FROM LIQUID SAMPLES IN SMALL VOLUMES.

Technical field

The present invention is within the field of handling small liquid sample volumes and performing chemistry therewith. More specifically it relates to a method of preventing evaporation from the liquid sample in such operations, which method makes it possible to perform rapid and reliable operations in a simple way even with ultra-small sample volumes.

Background of the invention

There is a great need for technology to handle especially nano- to femtoliter sized volumes of liquid samples and reagents, and to perform chemistry (synthesis, biochemical assays, analysis etc.) within such volumes. This is *e.g.* necessary when only very small amounts of sample are available. Such samples can *e.g.* be valuable isolates (natural products or biological material) or products from any other origin. When keeping such samples in a very small volume, it will be possible to maintain a high concentration. This is of great importance for obtaining fast reaction kinetics. An analogy can be found in living cells, where extremely *small amounts* (*e.g.* attograms) of biocomponents (peptides/proteins) are present in *high concentrations* due to the small (in the order of femtoliters) volume of the cell. Thus, bioreactions can proceed at high speed. Another important feature of chemistry in ultra-small volumes is that the small amounts of chemicals employed in such systems allow the use of rare, expensive chemicals at a reasonable cost. Other advantages include the reduced bench space, needed for performing chemistry in small volumes. Additionally, environmental pollution and personal hazard risks from

chemicals (solvents etc.) are minimized, due to the low consumption of materials.

Several devices to perform chemistry in nL - pL sized volumes have been suggested. The fluid containers used are frequently capillary tubing (ref 1-5) or channels , produced by photolithography and subsequent etching in glass, quartz, silicon or polymers (ref 6-8). In such devices, reactions can be performed either statically (ref 3, 4) or in a fluid flow (ref 1, 2, 5-8). However, such containers are inherently closed systems, and it is difficult to add or remove liquids, particularly when low volume, very narrow tubes or channels are employed. Another approach is to use open vials so that the dispensing and sampling of liquids/reagents is facilitated. Such vials can be produced in different materials like polymers, glass, silicon, quartz etc. Manufacturing procedures can range from making simple indentations in plastic with a tiny hard object (a pin) to advanced lithographic processes and subsequent isotropic or anisotropic etching (ref 9, 10). Chemistry can also be performed in tiny sample droplets , placed on a flat surface in a defined position. A schematic view of a typical structure with a multiple number of microvials (ref 11) , anisotropically etched in silicon will be shown later on in figure 1.

The lithographically produced structures have the advantage that a great number of vials (*e.g.* 100.000) can be made on a substrate with comparatively little effort. Thus, a large number of chemical operations (reactions) could be carried out on *e.g.* a silicon wafer. This is of great potential for high throughput screening in diagnostics or drug development, or in combinatorial chemistry, where a large number of chemical reactions or assays are performed in a systematic way.

A fundamental problem, related to the use of the open microvials or droplets on a surface as described above, is the evaporation of solvent in which the sample is dissolved . It is easy to understand that, due to the increased surface/volume ratio, nL - pL sized volumes of *e.g.* water will evaporate in a few seconds, if left exposed to an ordinary atmospheric surrounding. The evaporation can be prevented by covering the vials with membranes or "microlids" (ref 12). However, this method has drawbacks. Apart from the technically complicated miniaturized arrangement, small amounts of material can easily adhere to the cover. This can lead to cross contamination between vials and/or loss of material during opening and closing.

Another method is to impair the evaporation by saturation of the surrounding air above the vials or the surface with the solvent. However, this is difficult to control, and it has been found in practice that evaporation from the vials cannot be effectively avoided. Also, an operation near the boiling point of the solvent will make this approach impractical. A further drawback of this approach is that the vials have to be enclosed in a gas tight surrounding, to allow a saturation of the air above the vials with solvent. This impairs the access to the vials with dispensing devices, such as capillary tubing, syringes etc.

An additional technique to deal with the mentioned problem of evaporation is to continuously add solvents to the nL - pL sized vials, or to droplets on a flat surface. As long as the temperature of the vial (and the solvent within it) is moderate in relation to the boiling point of the solvent (*e.g.* 30-40°C for water solutions), this is a satisfactory technique. Even when using an array of vials, it is possible to use a matching array of solvent supplying capillaries , and the solvent level in

the individual vials can be held constant for several hours (this has *e.g.* been performed by the inventors with a row of eight vials and capillaries during 2-4h at 37°C). A schematic of the process will be shown below in figure 2.

However, this technique has also several drawbacks. One requirement is that the solvent, which is continuously added to the vial must be of very high purity, since impurities are continuously concentrated and accumulated from the evaporating solvent. Furthermore, it is difficult to operate at temperatures approaching the boiling point of the solvent. For example, if water is used as a solvent, and the temperature is raised to 90 °C in the vial, it is no longer possible to compensate for the evaporation in a controlled manner. In fact, the practical limits for this case, as evaluated in our laboratory, showed to be 70 °C.

Still another technology is utilized, to prevent evaporation of small amounts of solvent from miniature containers. This is by covering the solvent/sample with a thin layer of oil or molten wax, floating on top of the sample. As this liquid does not evaporate at the relevant operating temperature, a shield of the oil is formed which prevents evaporation of the sample A typical application is found in commercially available PCR reactors, where water based sampled are temperature- cycled between 60 and 95 °C. The sample is covered with a mineral oil during the temperature cycles, to prevent evaporation of water from the sample. However, for samples of very small volume (nL - pL), this technology is not useful in practice, because it becomes difficult to separate the oil from the sample after the reaction has been completed. Attempts to do this have either failed or have been associated with very large sample losses.

General description of the invention

According to the present invention a new technology is proposed to prevent evaporation of liquid from a small volume sample during any handling or chemical operation thereof. The basic principle is that during or in connection with the operation referred to the liquid sample is covered with a volatile liquid which is immiscible with the sample liquid.

In this context "covered with" means that said volatile liquid (covering liquid) is of such a density relative to the liquid sample that it floats on top of the sample and acts as a liquid lock, or if a reversed geometry is used, the liquid sample floats on top of the cover liquid.

As to the term "volatile liquid" this means that it evaporates or can be evaporated from the liquid sample without interfering or interacting with or decomposing any of the components thereof, i.e. neither the ingredients of the sample nor the solvent used. Furthermore, it should be completely evaporable from the liquid sample after the operation has been finalized, also in this case without decomposing or negatively influencing upon the same. In other words the cover liquid is selected so as to be volatilizable or evaporable at a temperature below the decomposition temperature of the components of the liquid sample. Moreover it should preferably be evaporable already at the temperature at which the operation is performed, as will be described more in detail below. In this connection it should be understood that "volatile" is used in the common sense meaning vaporizing or evaporating quickly, or similar, and that the operation referred to, and thereby the method claimed, is performed (well) below the decomposition temperature of the compo-

nentns of the liquid sample. Expressed in another way the term "evaporate" has the common meaning to drive out or draw off in the form of vapour. In this context it should also be added that also the time aspect is of course to be considered to avoid decomposition of the components of the liquid sample. That is, even if working below said decomposition temperature (where generally normal or atmospheric pressure is referred to) biological samples are often so sensitive that prolonged exposure to such temperature will have a negative impact on the sample. In other words temperature and time conditions are to be chosen so as to avoid the decomposition referred to. Thus, e.g. use of temperatures below said decomposition temperature (such as under vacuum) for oils or other non-volatile liquids will require such prolonged exposures that the sample will be negatively affected.

With reference to the term "small volume sample", this includes any of the previously known small volume operations, such as micro or nano litre operations. However, the new technology presented broadens the small volume operational range to include also the nano to femto litre range. In other words, an especially valuable aspect of the invention is that the method can be performed in a simple, rapid and reliable way also in the range of nano to femto litre volumes, e.g. pico to femto litre volumes. The technique might even be refined so as to enable operations with even smaller volumes, for instance atto litre volumes.

The handling or chemical operation referred to can be any of those operations which have previously been used in connection with small volume samples. Thus, as was mentioned above the chemical operation can be e.g. a synthesis, a biochemical assay or an analysis operation and the method is extremely well suited for use in connection with liquid samples containing biomolecules. Such

samples are generally very sensitive to higher temperature levels and temperature changes as well as to prolonged exposure to even moderately elevated temperatures and since the new technique according to the invention is very versatile as to temperature control levels it can be tailored for any biomolecular sample. Thus, it can be performed in minutes or even in seconds, for instance merely by discontinuing the feed of covering liquid. Examples of biomolecular samples to which the present method is especially applicable are peptides, proteins (e.g. enzymes) and nucleic acids (e.g. DNA).

Detailed description of the invention

More specifically, the present invention relates to a method of preventing evaporation from a small volume liquid sample, especially an ultra-small volume liquid sample, during any handling or chemical operation thereof, which comprises performing said operation with the sample covered by a layer of a liquid which is substantially immiscible with said liquid sample and which is evaporable at such temperature and time conditions at which decomposition of the components of the liquid sample is avoided.

According to an especially interesting embodiment of the method claimed the covering liquid is volatile, or evaporable, already at the temperature at which the operation in question is performed. A great advantage with such a choice of cover liquid thus is that this enables a rapid, complete evaporation of said cover liquid once the operation has been terminated making the sample immediately ready for the next step without any complicated or time-consuming separation being needed. This is extremely important for most biomolecules as they are very sensitive to prolonged exposure to elevated temperatures. Thus, such short times as less than 1 minute, or less than 30

seconds or even less than 20, 15 or 10 seconds, to evaporate the covering liquid may be applicable in optimum cases. Otherwise, evaporation times less than 30 minutes, such as less than 20, 10 or even 5 minutes, can be regarded valuable contributions to this specific technical field.

To compensate for the cover liquid which is evaporated during the operation in such a case supplementary or additional cover liquid is added during the operation. Said addition can be performed intermittently or continuously, continuous addition or feed being preferred.

According to one preferable embodiment of the method said additional covering liquid is added at substantially the same rate as the evaporation rate thereof as this means a smooth and steady state operation.

According to another embodiment of the invention, however, the additional covering liquid is added in an amount in excess of the amount evaporating from the sample, the appropriate layer thickness of covering liquid above the sample being controlled via any conventional overflow system. Thus, in this way the covering liquid also acts as a protection shield against the surrounding, for instance to continuously remove dust attracted from the surrounding by the overflow of cover liquid.

As should be obvious to a person skilled in the art the sample is generally present in a plurality of individual containers, vials or droplets, etc., and a preferable embodiment of the invention is here represented by a simple technique where a common supply (and preferably also a common outlet) of covering liquid is utilized for a number of individual liquid samples.

As to the nature of the covering liquid, in addition to the properties or characteristics referred to above, said covering liquid is also preferably chosen so as to be evaporable at a temperature level not too high above

the operation temperature referred to and preferably relatively close thereto. Generally this means that the boiling point of the covering liquid is at most 100°C above the temperature at which the operation is performed. Preferably it is at most 80°C above and more preferably at most 50°C above said operation temperature.

Furthermore, said boiling point is generally chosen so as to be above, preferably at least 5°C above, said operation temperature as otherwise the covering liquid will be continuously boiling away. More preferably said boiling point is at least 10°C above the operation temperature. According to another embodiment of the invention the covering liquid is chosen so as to be fully evaporable below 100°C and preferably below 80°C. In many cases, however, the sample may be sensitive also to such high temperature levels and consequently said cover liquid then may have to be evaporable already below 60°C or even below 50°C.

Generally the covering liquid is chosen so as to be substantially inert relative to the sample and also preferably relative to the reaction products from the sample.

In some cases it can, however, be interesting to use a cover liquid which interacts with reaction products, e.g. waste products (gases, etc.), from the sample.

In other cases it can also be of value to include one or more of the reactants to be used in the operation in said covering liquid to feed the same, even sequentially, to the reaction.

Still another embodiment of the invention is represented by the case where the covering liquid is chosen so as to have the ability of extracting one or more components from the sample.

One preferable group of covering liquids is alkanes having appropriate boiling points, e.g. alkanes, especially n-alkanes, having 5-10 carbon atoms, such as pentane, hexane, heptane, octane, nonane and decane. In this context is should be understood that the term cover liquid also includes any mixtures(s) of liquids. As a guidance for the invention it could also be mentioned that the boiling points of the preferred alkanes having 5-8 carbon atoms are as follows (for the n-alkanes):

pentane	+36°C
hexane	+69°C
heptane	+98°C
octane	+126°C

In many instances, even more preferred covering liquids are (per)fluorocarbons, which are known for their minimal interference with biomolecules, similar considerations being made concerning boiling point ranges as in connection with the alkanes or other appropriate group of covering liquids.

After the chemical reaction, the biochemical assay or any other operation has been completed, the supply of cover liquid is discontinued. As a consequence, the cover liquid will generally disappear completely by evaporation within a short period of time (depending on the boiling point of the cover liquid and the temperature of the liquid). Subsequently, if desired, the solvent from the sample, now uncovered, will also disappear, and a dry sample is left in the microvial (or a very small, dry sample spot, if a flat surface is employed as a "container"). The reaction products can now be dissolved in new solvent, or be utilized *in situ*, in its solid state, for subsequent analysis (*e.g.* infrared spectroscopy, MALDI mass spectrometry (ref 13) etc.). An example of a possi-

ble operational sequence will be described later on in connection with Figure 4.

Some particular features and advantages, (particularly showing the increased degrees of freedom) of the invention can thus be summarized as follows:

* Evaporation of a solvent from the vials (or solvent droplets on a surface) is effectively minimized (only very small amounts of the solvent are dissolved in the cover liquid. This may be most pronounced at higher temperatures).

* The cover liquid can be simply and completely removed by natural or forced evaporation.

* The operating temperature can be very close to the boiling point of the sample liquid.

* Full accessibility to the reaction liquid is maintained at all time, since there is no mechanical lock. It is *e.g.* possible to add reagents or remove fluid from the sample during a reaction or incubation, while the reaction mixture is covered with covering liquid.

* If the covering liquid is added in excess during the reaction, dust attracted from the surrounding is continuously removed by the overflow of the cover liquid. The cover liquid is therefore not only a barrier against evaporation. It also acts as a dynamic, protective shield against the surrounding.

* In setups, where a plurality of individual vials or droplets on a surface are utilized, to perform multiple reactions (on a chip, glass plate etc.), only one common supply of covering liquid can be utilized, instead of feeding solvent to individual vials by an array of capillary tubes. This greatly facilitates the setup, particularly when using a matrix and/or a large number of vials.

* Full freedom is maintained to choose the properties of the cover liquid, as long as this liquid is not miscible with the sample liquid. *E.g.* for low temperature operation, a relatively volatile liquid can be chosen. For high temperature operations, a less volatile liquid should preferably be used.

* Volatile covering liquids are often of low viscosity, in contrast to high boiling oils . This facilitates penetration through the cover layer, reduces the "stickiness" and counteracts the formation of emulsions.

*The flowing covering liquid may contain reagents as well. In this way a reagent can be supplied to the vials. Alternatively the covering liquid may have properties to extract components from the sample. Also a procedure, where several reagents are sequentially added to the flow of covering liquid can be envisioned.

After the reaction has taken place, and the covering solvent, and if desired also the sample solvent has evaporated, the reaction products can be re-dissolved in an aliquot of the original or a different solvent to be able to remove the sample from the vial or the surface for subsequent analysis or other purposes. One suitable way of operation is to add the solvent via a capillary tube in a continuous way (according the principles shown in figure 2), while the sample can be withdrawn with another capillary tube. A particular example of this procedure will be shown in figure 7, where water is used as a solvent.

Likewise, other sequential procedures can be adopted. For example, a dry sample can be placed in a vial or on a surface, followed by the application of the covering liquid. The liquid (containing possible reagents) , which dissolves the sample is added on top of the covering liquid. This liquid then positions itself

under the covering liquid, due to gravitational forces, thereby dissolving the sample. It is also possible to start with the application of a covering liquid, through which the sample (and subsequently reagents) are applied.

A particular way of dosing reagents , solvent or dissolved sample is in the form of tiny droplets of high velocity, generated by the same principle as in inkjet computer printers. Such devices are commercially available for dispensing microdrops of various solutions (ref 14) Due to the high velocity of the droplets, a penetration of the cover layer is rapidly achieved (the sample is "shooting" through the surface cover layer). This is facilitated by adjusting the thickness of the cover layer, which in its turn is adjusted by the supply of liquid and the evaporation rate of the same liquid. The "ink-jet" principle is also useful for a periodical make-up of sample liquid, to compensate for possible liquid losses, incurred during long term operation and/or at elevated temperatures.

Figures

The invention, as well as some prior art, will now be further described in connection with the drawings which show the following:

Fig. 1 shows a schematic view of an anisotropically etched vial in silicon representing the prior art;

Fig. 2 shows a schematic prior art process with continuous addition of solvent to a small volume sample;

Fig. 3 shows schematically an embodiment of the method according to the invention with continuous addition of cover liquid;

Fig. 4 shows one embodiment of an operational sequence of the method according to the invention;

Fig. 5 shows the principle of removing fluid from the sample during the method according to the invention;

Fig. 6 shows an embodiment of the method according to the invention where covering liquid is added in excess;

Fig. 7 shows an embodiment of the method claimed where solvent is added and sample is withdrawn after the operation has been discontinued;

Fig. 8 shows a holder to be used for the mounting of silicon-chip based vials in a method according to the invention; and

Fig. 9 shows the results from a capillary electrophoretic column analysis of a reaction mixture.

More specifically Fig. 1 shows an etched vial in silicon as disclosed e.g. in reference 2. In such a vial device L is typically 25-400 μm.

Fig. 2a shows a liquid sample in the form of droplet 1 on a flat surface and Fig. 2b a liquid sample 2 in a vial where in both cases in accordance with known technique solvent is continuously added at the same feed rate V/t as the evaporation rate V/t for the sample liquid (V=volume; t=time).

Fig. 3a shows a vial with a liquid sample 2 covered by a cover liquid 3 being added continuously via a capillary tube 4, the feed rate V/t for said additional cover liquid being the same as the evaporation rate V/t of said cover liquid 3.

Fig. 3b shows a schematic view of a device 5 for continuous feed, via a common inlet 6, of cover liquid to a number of individual vials with liquid samples 2 to prevent evaporation therefrom. In this case the supply rate is V_{1+2}/t while the evaporation rate is V_1/t, i.e. an excess of cover liquid is used, and an overflow 7 being used, the overflow rate being V_2/t.

Fig. 3c schematically shows the principle of using a reversed geometry with sample liquid flowing on top of cover liquid. Thus, with the same meanings of the refer-

ence numerals as in the other figures a reversed chip is shown with sample liquid 2 in a vial and cover liquid 3 therebelow.

The sequence shown in Fig. 4 comprises 8 steps where step 1 shows the starting vial with sample liquid, which is then evaporated to a dry sample in step 2, steps 1 and 2 being repeated for all reagents needed. In step 3 cover solvent and in step 4 sample solvent are added, while step 5 shows the reaction step.

In step 6 evaporation is then performed to dry reaction products, while in step 7 new cover solvent is added and in step 8 new solvent is added to dissolve reaction products for further operation thereof.

Fig. 5 shows the principle for removing sample 2 from a vial via a capillary tube 8 during the reaction.

Fig. 6 is similar to Fig. 3b but specifically shows the action of the cover liquid as a dust shield, dust particles 9 from the surrounding being continuously removed by the excess of cover liquid drained via the overflow 7.

Fig. 7 shows the principle for redissolving a sample with a supply of water and subsequent sample withdrawal. Since there is an equilibrium between surface evaporation and supply of water, the volume of the sample can be kept at a constant level. When this situation is reached, part of the sample can be withdrawn, e.g. with a capillary tube (right-hand-side tube).

Figs. 8 and 9, finally, will be described more in detail in connection with the working examples.

EXAMPLES

The invention will now be exemplified by some working examples representing a few embodiments of the invention.

Example 1

A reaction was performed between a protein (myoglobin) and a proteolytic enzyme (trypsin). When these two compounds are brought together under specific conditions (temperature, pH), the protein is digested by the enzyme and distinctly fragmented into a number of peptides. An analysis of these fragments provides a kind of fingerprint of the protein. This procedure is therefore an often used method for identification of proteins.

In this particular example, anisotropically etched silicon-chip based vials of the type previously shown in Fig. 1 were employed. The chip was mounted in a stainless steel holder, which is shown in Fig. 8, Fig. 8a showing a side view and Fig. 8b showing a top view thereof. The holder 10 contained a heating element 11 and a temperature sensor (not shown), in order to allow the reaction to proceed under controlled temperature. An internal channel 12 in the holder also provided the possibility to cool the holder. The chip was mounted in a recessed area 13 on top of the holder. In said recess the temperature sensor referred to can also be positioned. The recessed area was accessed by in- and outlet holes 14 and 15 for the covering liquid, which in its turn were connected to in- and outlet side entries in the holder.

In this way, a controlled flow of covering liquid could be administered, to cover the entire silicon chip, while keeping a balance between supply and evaporation of the covering liquid. The holder was mounted on a XY servo motor-controlled table. Samples and reagents were added from a container under controlled pressure or from a flow-controlled micro-pump, via narrow bore capillary tubes (ID 40 µm OD 105 µm), which could be precisely po-

sitioned with a motorized device near the square opening of a vial.

A mixture of myoglobin (8 mg/ml) and water-based buffer solution (NH_4HCO_3 8 mM pH 7.9) was dosed in eight individual, parallel operated 15 nL-sized vials. Thereafter, the water was allowed to evaporate, which occurred in about 40 sec. Octane was used as a covering liquid, and was guided over the chip surface from a pressurized container via the inlet hole in the recessed stainless steel surface. The excess of octane was removed by controlled suction via the drainage hole. When the octane level was stabilized, 15 nL of trypsin dissolved in water (0.2 mg/ml) was added to the individual vials. Thus, the dry myoglobin and buffer salts attached to the walls of the vials were dissolved, which started the digest reaction. To achieve optimal conditions for the reaction, the device was heated to 37 °C utilizing the build-in heating element and sensor. The temperature was controlled with a PID regulator.

The reaction was sustained under 2 hrs. During this time, the level of the buffer solution was monitored under a microscope. No detectable reduction in volume could be observed during this period, which demonstrates the efficiency of the cover liquid

Samples from the reaction mixture were periodically withdrawn by means of capillary tubes or vacuum injected directly into a capillary electrophoretic column. After completion of the reaction, the flow of octane was discontinued and the octane as well as the water of the sample evaporated. The dried sample was re-dissolved in water and the reaction mixture was analysed by capillary electrophoresis. The results thereof are shown in figure 9. The different peptide fragments, obtained during the reaction can be clearly distinguished.

The details of Fig. 9 are as follows:

CE (Capillary Electrophoretic) conditions; capillary length 85 cm (60 cm to detection), separation buffer 0.01 M KH_2PO_4pH 7.0, 15 kV, 6 µA, on-column detection 210 nm.

a) upper electropherogram; CE analysis of sample injected through the cover layer after 45 min reaction time

b) lower electropherogram; CE analysis of redissolved sample after 2 hrs reaction time

Example 2

In this example a test to investigate the efficiency of the covering liquid under conditions near the boiling point of the sample liquid was carried out. The entire procedure was monitored under a microscope. First, an octane layer was formed on top of a silicon chip with empty vials (according to the procedure described above). Then, 15 nL of water, containing E120 (carminic acid; a dye to facilitate observation of the water phase) was injected through the octane layer via a glass capillary. After this step, the temperature was raised from 25 °C to 95 °C. Once this temperature was established, the temperature was brought down to 72 °C. The cooling time was 0.5 min. Then the temperature was raised again to 95 °C, which took 2.5 min. Thus, the total cycle time of this procedure was 3 minutes.

After seven such cycles (a total of 18 minutes), it was possible to notice a slight decrease of the water level. However, the level of the water was easily reestablished by injection of some extra water via a capillary tube (injection of water would have been still simpler by using the ink-jet like device mentioned earlier). The temperature cycling process could be repeated for more than 20 times, with only an occasional need for water addition.

The above results show, that the present invention

makes it possible to operate under conditions near the boiling point of the solvent, without suffering from an excessive evaporation, yet leaving a full degree of freedom to add or remove liquids during the reaction. This would not be possible with constructions based on a mechanical lid or any of the other concepts, described above, without suffering from substantial component losses or inconveniences and/or contaminations from oils or similar. Using the volumes described, water would evaporate from open structures in less than a second already at a temperature around 60 °C. The cyclic heating/cooling experiment, described above also shows that the present invention would make it feasible to perform a large number of individual, parallel operated PCR reactions in nanoliter - picoliter sized containers.

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CLAIMS

1. A method of preventing evaporation from a small volume liquid sample, especially an ultra-small volume liquid sample, during any handling or chemical operation thereof, which comprises performing said operation with the sample covered by a layer of a liquid which is substantially immiscible with said liquid sample and which is evaporable at such temperature and time conditions at which decomposition of the components of the liquid sample is avoided.

2. A method as claimed in claim 1, wherein the covering liquid is chosen so as to be evaporable at the temperature at which said operation is performed.

3. A method as claimed in claim 2, wherein additional covering liquid is added, preferably continuously, during the operation to compensate for evaporating liquid covering the sample.

4. A method as claimed in claim 3, wherein said additional covering liquid is added at substantially the same feed rate as the evaporation rate of the liquid covering the cample.

5. A method as claimed in any one of claims 1-3, wherein said additional covering liquid is added in an amount in excess relative to the amount of covering liquid evaporating from the sample, an overflow system being used such that said covering liquid also acts as a protective shield against the surrounding.

6. A method as claimed in any one of claims 2-5, wherein a common supply of covering liquid is utilized for a number of individual liquid samples.

7. A method as claimed in any one of the preceding claims, wherein the covering liquid is chosen so as to have a boiling point at least 5°C above, preferably at

least 10°C above, and at most 100°C above, preferably at most 80°C above, such as at most 50°C above, the temperature at which said operation is performed.

8. A method as claimed in any one of the preceding claims, wherein the covering liquid is chosen so as to be fully evaporable below 100°C, preferably below 80°C, e.g. below 60°C or below 50°C.

9. A method as claimed in any one of the preceding claims, wherein said covering liquid is chosen so as to be substantially inert relative to the sample and reaction products therefrom.

10. A method as claimed in any one of claims 1-8, wherein said covering liquid is chosen so as to interact with reaction products, e.g. waste products, from the sample.

11. A method as claimed in any one of claims 1-8 and 9, wherein said covering liquid contains reagent(s) to be used in said operation.

12. A method as claimed in claim 11, wherein said covering liquid contains two or more reagents which are added sequentially to the sample.

13. A method as claimed in any one of the preceding claims, wherein said covering liquid is chosen so as to have the ability of extracting component(s) from the sample.

14. A method as claimed in any one of the preceding claims, wherein said covering liquid is selected from alkanes, especially n-alkanes, preferably having 5-10 carbon atoms, such as pentane, hexane, heptane, octane, nonane and decane and mixtures thereof.

15. A method as claimed in any one of claims 1 - 13, wherein said covering liquid is selected from (per)fluorocarbons.

16. A method as claimed in any one of the preceding claims, wherein said operation is performed with the

liquid sample in ultra-small open container(s) or vial(s).

17. A method as claimed in any one of claims 1-15, wherein said operation is performed with the liquid sample in the form of ultra-small droplets on a substantially flat surface.

18. A method as claimed in any one of the preceding claims, wherein said ultra-small volume is within the range of nano- to femtolitre.

19. A method as claimed in any one of the preceding claims, wherein said sample is selected from biomolecules, especially peptides, proteins, e.g. enzymes, and nucleic acids, e.g. DNA.

20. A method as claimed in any one of the preceding claims, which comprises discontinuing the addition of additional covering liquid, where appropriate, and evaporating all covering liquid when said operation has been terminated.

Figure 1

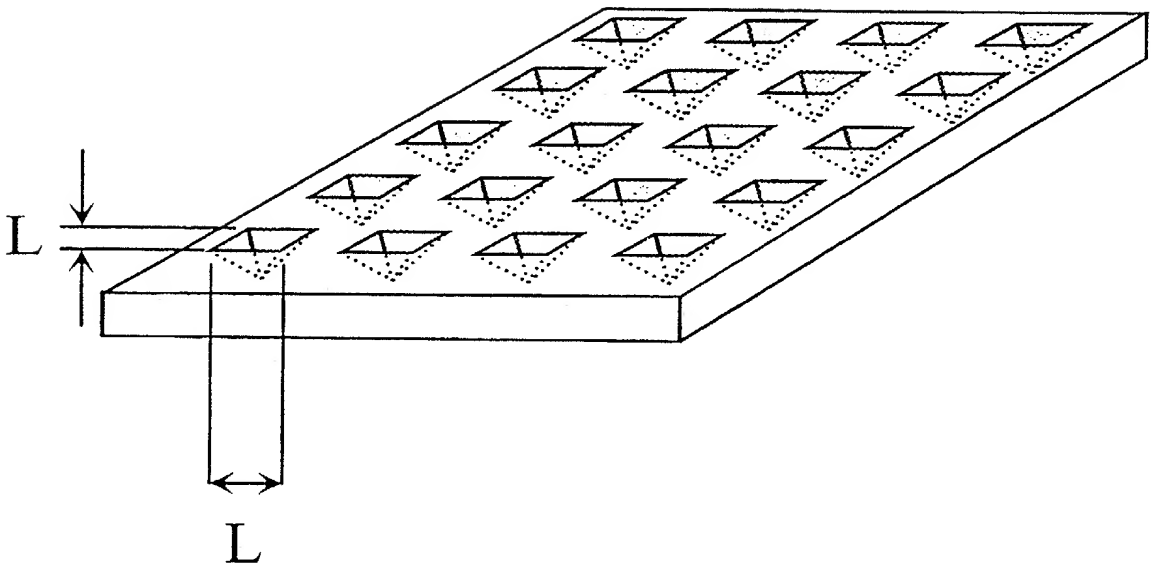


Figure 2a

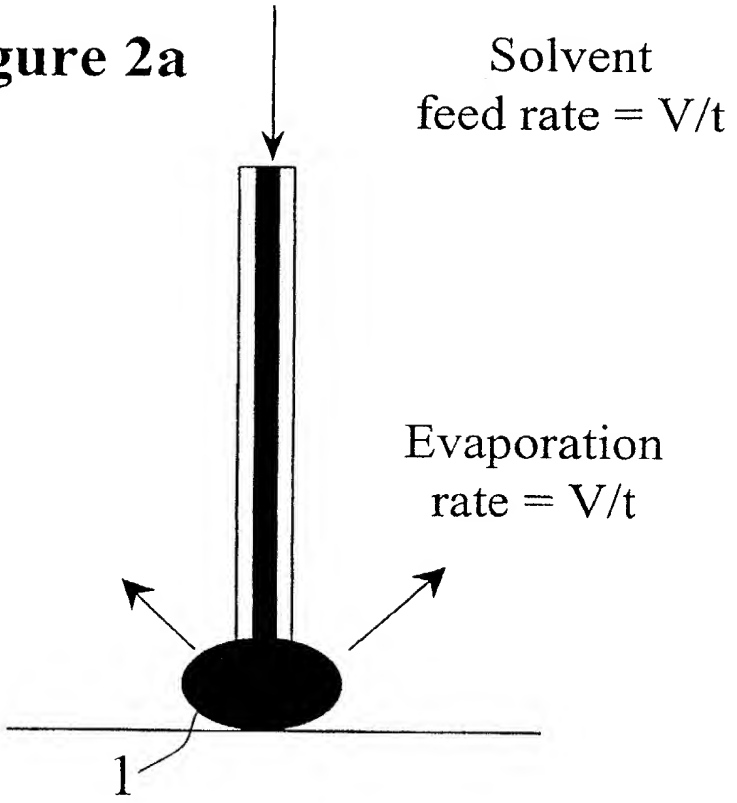


Figure 2b

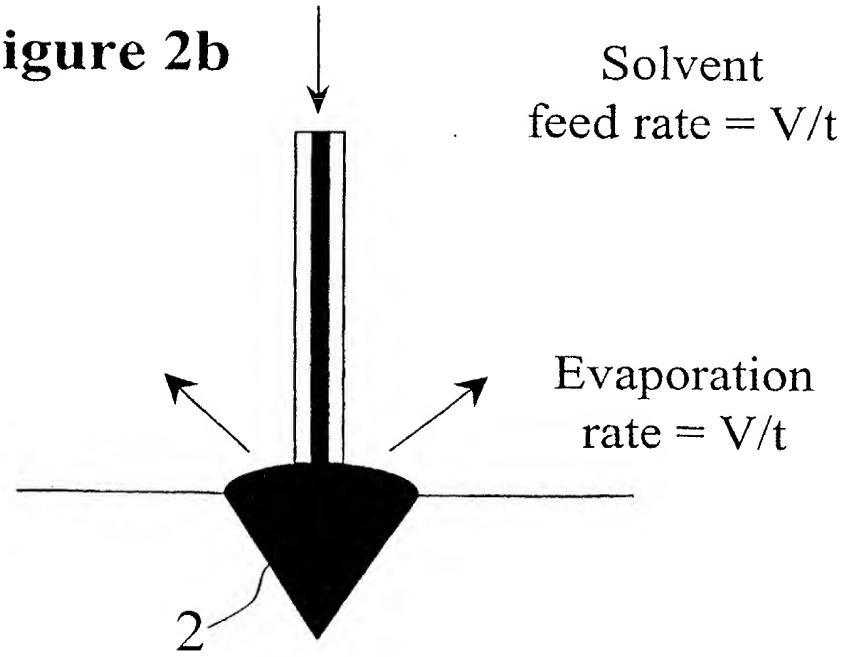


Figure 3a

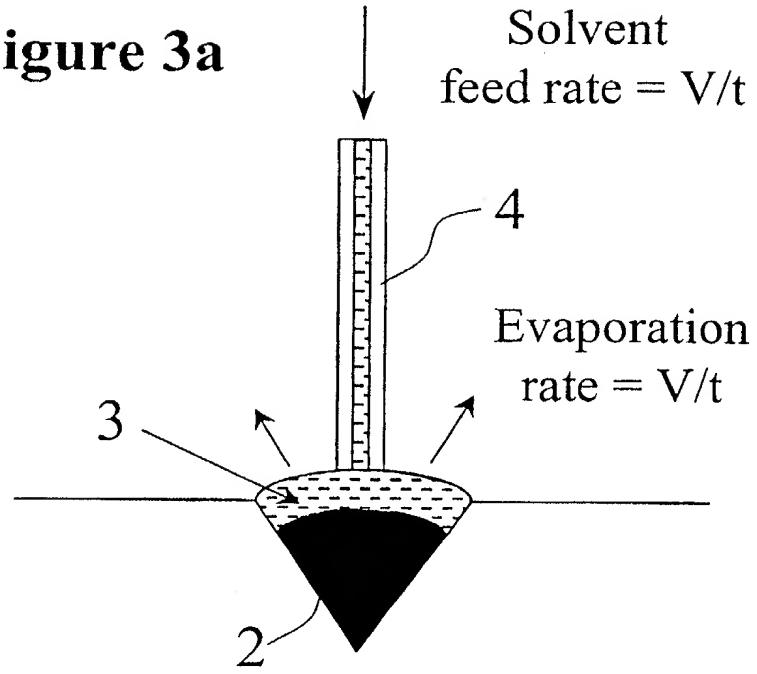


Figure 3b

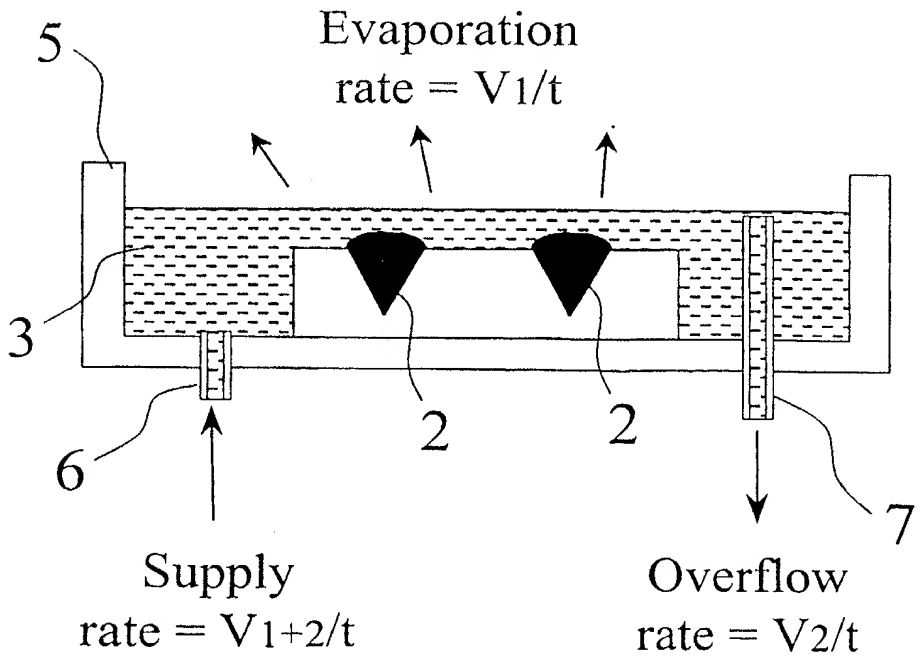


Figure 3c

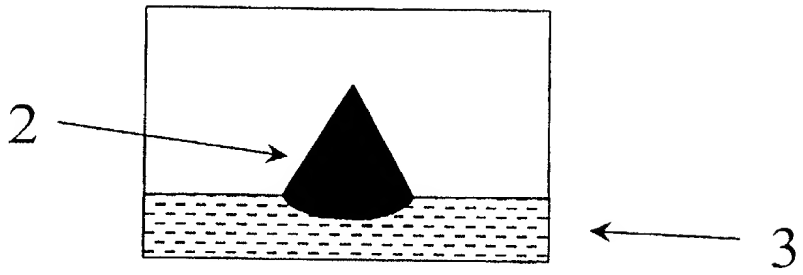
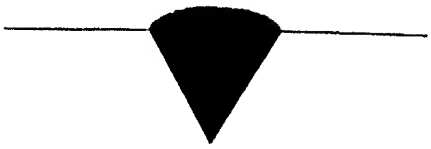
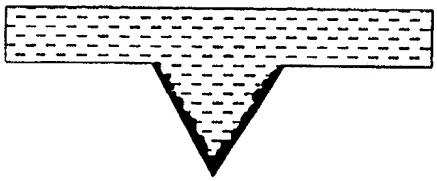


Figure 4

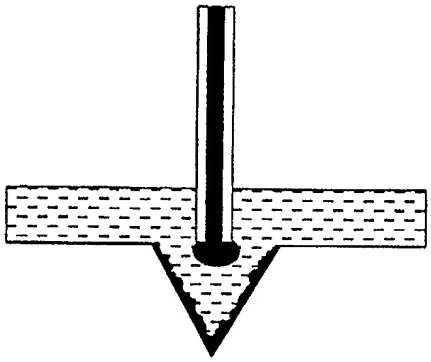


Step 1: Sample liquid

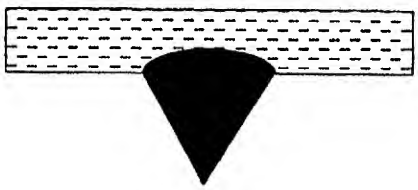
Step 2: Evaporate -> dry sample
Repeat step 1 and 2 for all reagents needed.



Step 3: Cover solvent

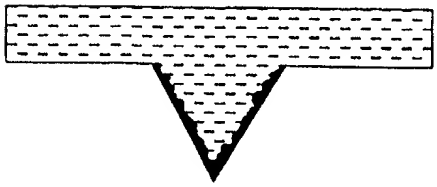


Step 4: Sample solvent

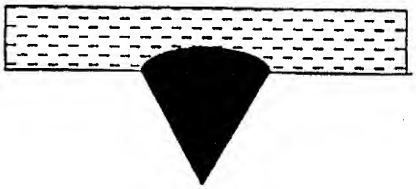


Step 5: Reaction

Step 6: Evaporate ->
-> dry reaction products

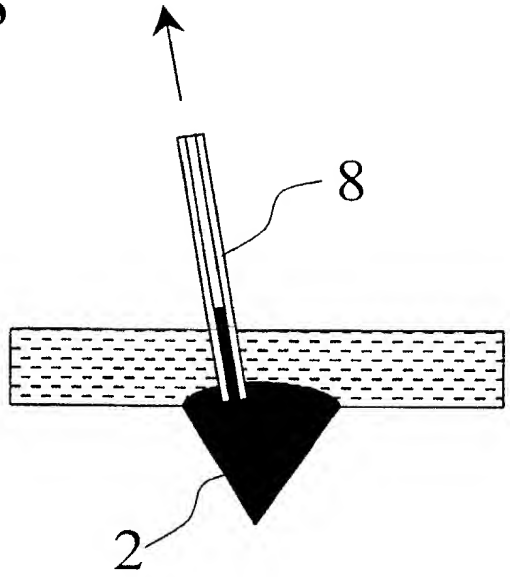


Step 7: Cover solvent



Step 8: New solvent to dissolve reaction products

Figure 5



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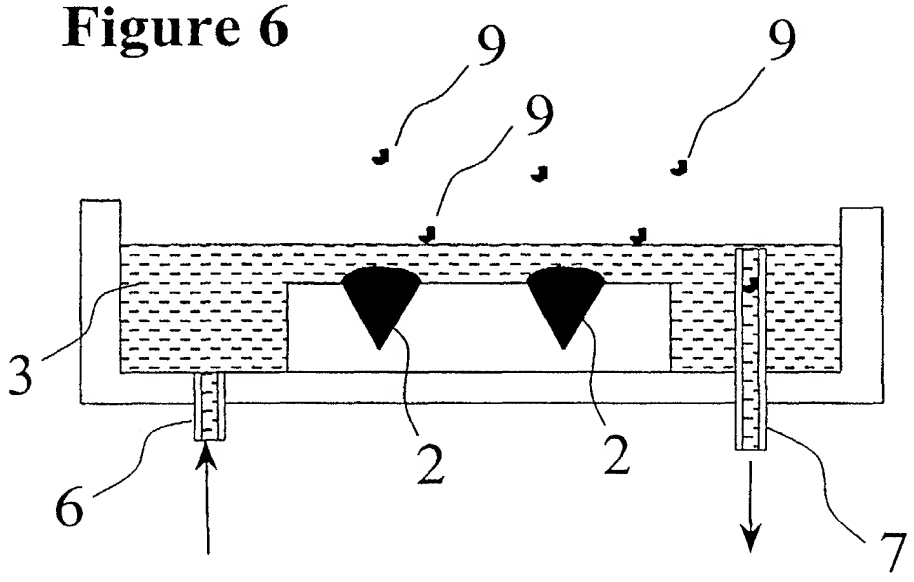


Figure 7

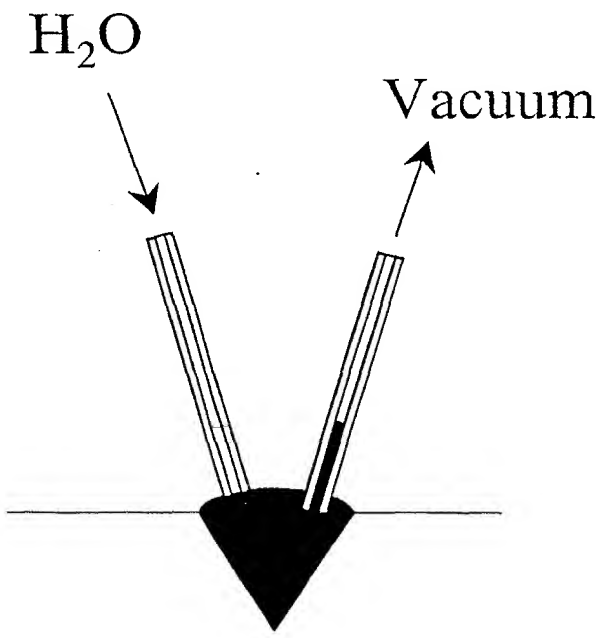


Figure 8a

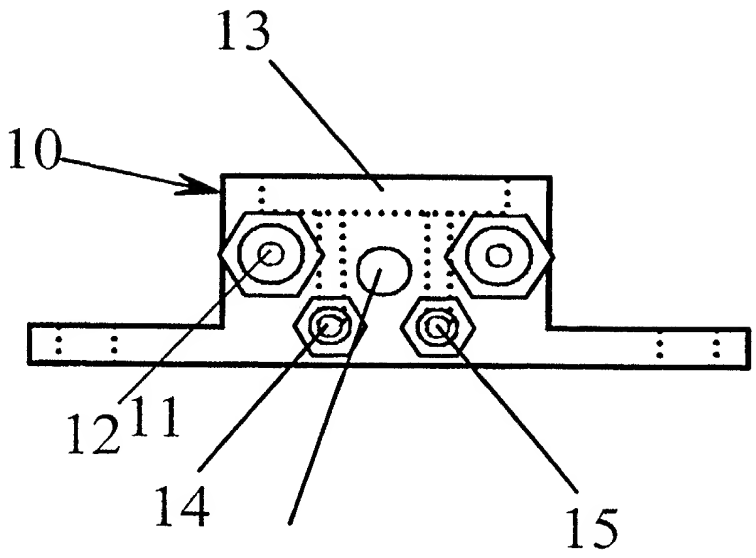


Figure 8b

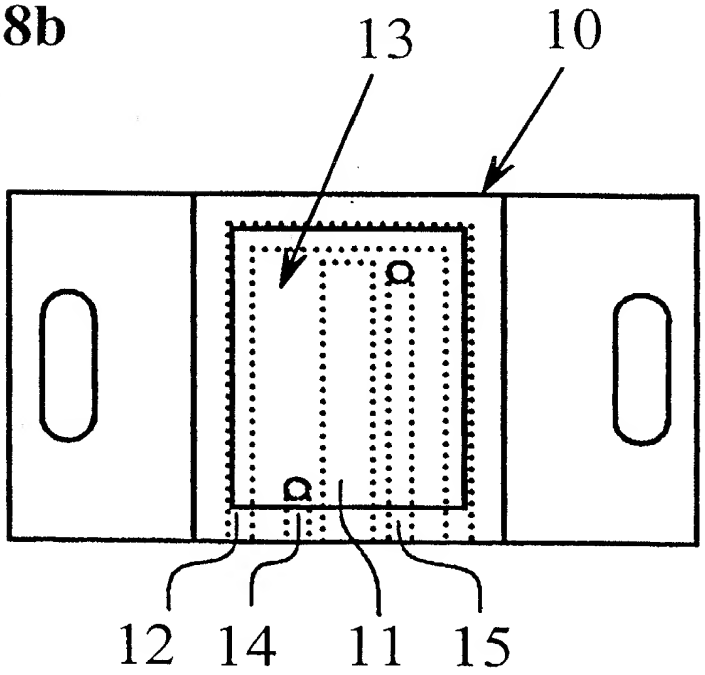
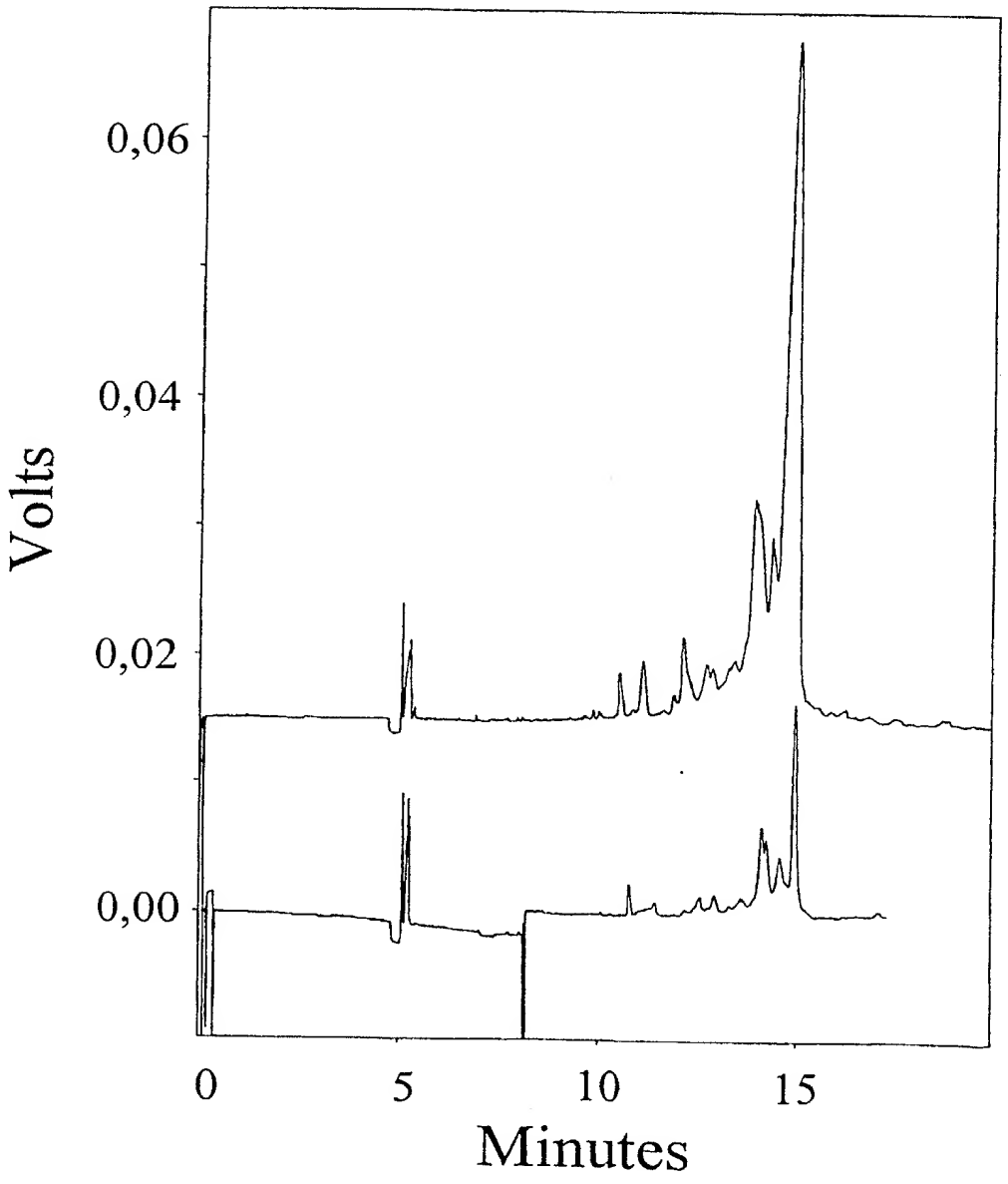


Figure 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00095

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 1/28, G01N 1/30
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5225325 A (PHILLIP C. MILLER ET AL), 6 July 1993 (06.07.93), column 6, line 35 - column 7, line 49	1,14
A	US 5549848 A (RON ZEHEB ET AL), 27 August 1996 (27.08.96), column 6, line 55 - column 7, line 13	1-20
A	EP 0159603 A2 (ABBOTT LABORATORIES), 30 October 1985 (30.10.85), page 3, line 34 - page 4, line 25	1-20

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1998	04-05-1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Ulla Granlund Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

Information on patent family members

02/04/98

International application No.
PCT/SE 98/00095

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